

AMENDMENTS TO THE CLAIMS

Please amend the claims as noted below, without prejudice to subsequent renewal. The listing of claims below replaces all prior versions, and listings, of claims in the application.

These amendments introduce no new matter and support for the amendment is replete throughout the specification and claims as originally filed. These amendments are made without prejudice and are not to be construed as abandonment or dedication of the previously claimed subject matter, or agreement with any objection or rejection of record.

Listing of Claims:

1-25. (Cancelled)

26. (Currently amended) A composition, comprising:

a cell comprising an enzyme and a caged sensor for detecting an activity of the enzyme, which caged sensor comprises:

a) one or more molecules collectively comprising:

i) a substrate for the enzyme, wherein the substrate is in a first state on which the enzyme can act, thereby converting the substrate to a second state, and

ii) a first label, wherein a first signal exhibited by the first label when the substrate is in its first state is distinguishable from a second signal exhibited by the first label when the substrate is in its second state; ~~and~~,

b) one or more first caging groups associated with the one or more molecules, the first caging groups inhibiting the enzyme from acting upon the substrate; and,

c) optionally, one or more second caging groups associated with a phosphobinder;

wherein the enzyme is a protein kinase that phosphorylates tyrosine, serine and/or threonine;

wherein the one or more molecules comprise one polypeptide; wherein the one polypeptide comprises the substrate for the kinase and the first label and comprises a second label or a quencher; wherein the first label and the second label or the quencher interact to produce the first signal when the substrate is not phosphorylated; and wherein phosphorylation of the substrate prevents the interaction of the first label and the second label or the quencher, thereby resulting in production of the second signal;

wherein phosphorylation of the substrate triggers a conformational change in the polypeptide, the conformational change preventing the interaction of the first label and the second label or the quencher; or wherein phosphorylation of the substrate results in binding of the phosphobinder to the phosphorylated substrate, the binding of the phosphobinder preventing the interaction of the first label and the second label or the quencher; wherein the presence of the second caging groups ~~which~~ prevents the phosphobinder from binding the phosphorylated substrate.

27. (Previously presented) The composition of claim **26** or **309**, wherein the second caging groups are removable under different conditions than the first caging groups preventing phosphorylation of the substrate.

28. (Currently amended) A composition, comprising:

a cell comprising an enzyme and a caged sensor for detecting an activity of the enzyme, which caged sensor comprises:

a) one or more molecules collectively comprising:

i) a substrate for the enzyme, wherein the substrate is in a first state on which the enzyme can act, thereby converting the substrate to a second state, and

ii) a first label, wherein a first signal exhibited by the first label when the substrate is in its first state is distinguishable from a second signal exhibited by the first label when the substrate is in its second state; ~~and~~,

b) one or more first caging groups associated with the one or more molecules, the first caging groups inhibiting the enzyme from acting upon the substrate; and,

c) optionally, a phosphobinder comprising an antibody, an SH-2 domain, a PTB domain, a 14-3-3 domain, an FHA domain, a WD40 domain and/or a WW domain;

wherein the enzyme is a protein kinase that phosphorylates tyrosine, serine and/or threonine;

wherein the one or more molecules comprise one polypeptide; wherein the one polypeptide comprises the substrate for the kinase and the first label and comprises a second label or a quencher; wherein the first label and the second label or the quencher interact to produce the first signal when the substrate is not phosphorylated; and wherein phosphorylation of the substrate prevents the interaction of the first label and the second label or the quencher, thereby resulting in production of the second signal;

wherein phosphorylation of the substrate triggers a conformational change in the polypeptide, the conformational change preventing the interaction of the first label and the second label or the quencher; or wherein phosphorylation of the substrate results in binding of the phosphobinder to the phosphorylated substrate, the binding of the phosphobinder preventing the interaction of the first label and the second label or the quencher.

29-32. (Cancelled)

33. (Currently amended) A composition, comprising:

a cell comprising an enzyme and a caged sensor for detecting an activity of the enzyme, which caged sensor comprises:

a) one or more molecules collectively comprising:

i) a substrate for the enzyme, wherein the substrate is in a first state on which the enzyme can act, thereby converting the substrate to a second state, and

ii) a first label, wherein a first signal exhibited by the first label when the substrate is in its first state is distinguishable from a second signal exhibited by the first label when the substrate is in its second state; ~~and~~,

b) one or more first caging groups associated with the one or more molecules, the first caging groups inhibiting the enzyme from acting upon the substrate; and,

c) one or more second caging groups associated with a phosphobinder, wherein the presence of the ~~phosphobinder~~ second caging groups prevents the phosphobinder from binding ~~on~~ the phosphorylated substrate;

wherein the enzyme is a protein kinase that phosphorylates tyrosine, serine and/or threonine;

wherein the one or more molecules comprise one polypeptide; wherein the one polypeptide comprises the substrate for the kinase and the first label; wherein the polypeptide comprises the phosphobinder and a second label or a quencher; wherein the first label and the second label or the quencher do not interact when the substrate is not phosphorylated, thereby producing the first signal; and wherein phosphorylation of the substrate results in intramolecular binding of the phosphobinder to the phosphorylated substrate, the intramolecular binding resulting in the interaction of the first label and the second label or the quencher, thereby producing the second signal; or, wherein the one or more molecules comprise a first polypeptide and a second polypeptide; wherein the first polypeptide comprises the substrate for the kinase and the first label; wherein the second polypeptide comprises the phosphobinder and a second label or a quencher; wherein the first label and the second label or the quencher do not interact when the substrate is not phosphorylated, thereby producing the first signal; and wherein phosphorylation of the substrate results in intermolecular binding of the phosphobinder to the phosphorylated substrate, the intermolecular binding resulting in the interaction of the first label and the second label or the quencher, thereby producing the second signal.

34. (Previously presented) The composition of claim **33** or **311**, wherein the second caging groups are removable under different conditions than the first caging groups preventing phosphorylation of the substrate.

35. (Currently amended) A composition, comprising:

a cell comprising an enzyme and a caged sensor for detecting an activity of the enzyme, which caged sensor comprises:

a) one or more molecules collectively comprising:

i) a substrate for the enzyme, wherein the substrate is in a first state on which the enzyme can act, thereby converting the substrate to a second state, and

ii) a first label, wherein a first signal exhibited by the first label when the substrate is in its first state is distinguishable from a second signal exhibited by the first label when the substrate is in its second state; ~~and~~,

b) one or more first caging groups associated with the one or more molecules, the first caging groups inhibiting the enzyme from acting upon the substrate; and,

c) a phosphobinder comprising: an antibody, an SH-2 domain, a PTB domain, a 14-3-3 domain, an FHA domain, a WD40 domain and/or a WW domain;

wherein the enzyme is a protein kinase that phosphorylates tyrosine, serine and/or threonine;

wherein the one or more molecules comprise one polypeptide; wherein the one polypeptide comprises the substrate for the kinase and the first label; wherein the polypeptide comprises the phosphobinder and a second label or a quencher; wherein the first label and the second label or the quencher do not interact when the substrate is not phosphorylated, thereby producing the first signal; and wherein phosphorylation of the substrate results in intramolecular binding of the phosphobinder to the phosphorylated substrate, the intramolecular binding resulting in the interaction of the first label and the second label or the quencher, thereby producing the second signal; or, wherein the one or more molecules comprise a first polypeptide and a second polypeptide; wherein the first polypeptide comprises the substrate for the kinase and the first label; wherein the second polypeptide comprises the phosphobinder and a second label or a quencher; wherein the first label and the second label or the quencher do not interact when the substrate is not phosphorylated, thereby producing the first signal; and wherein phosphorylation of the substrate results in intermolecular binding of the phosphobinder to the phosphorylated substrate, the intermolecular binding resulting in the interaction of the first label and the second label or the quencher, thereby producing the second signal.

36. (Previously presented) A composition, comprising:

a cell comprising an enzyme and a caged sensor for detecting an activity of the enzyme, which caged sensor comprises:

a) one or more molecules collectively comprising:

i) a substrate for the enzyme, wherein the substrate is in a first state on which the enzyme can act, thereby converting the substrate to a second state, and

ii) a first label, wherein a first signal exhibited by the first label when the substrate is in its first state is distinguishable from a second signal exhibited by the first label when the substrate is in its second state; and,

b) one or more first caging groups associated with the one or more molecules, the first caging groups inhibiting the enzyme from acting upon the substrate;

wherein the enzyme is a protein kinase that phosphorylates tyrosine, serine and/or threonine;

wherein the one or more molecules comprise one polypeptide; wherein the one polypeptide comprises the substrate for the kinase, a second substrate, the first label, a third label, a fourth label or a quencher, and a phosphobinder; the substrate comprising a serine, threonine, or tyrosine residue capable of being phosphorylated by the kinase; the second substrate being associated with one or more third caging groups, the presence of which prevents phosphorylation of the second substrate; wherein the first label is located at the serine, threonine, or tyrosine residue and exhibits the first signal when the residue is not phosphorylated and the second signal when the residue is phosphorylated; wherein the third label and the fourth label or the quencher do not interact when the second substrate is not phosphorylated, thereby producing a third signal; and wherein phosphorylation of the second substrate results in intramolecular binding of the phosphobinder to the phosphorylated second substrate, the intramolecular binding resulting in the interaction of the third label and the fourth label or the quencher, thereby producing a fourth signal, the fourth signal distinguishable from the first, second and third signals.

37. (Previously presented) The composition of claim **36** or **306**, wherein the second substrate is for the same kinase or for a different kinase.

38. (Previously presented) The composition of claim **36** or **306**, wherein the one or more third caging groups are located on a residue that can be phosphorylated by the kinase.

39. (Original) The composition of claim **38**, wherein the third caging groups preventing phosphorylation of the second substrate are removable under different conditions than the first caging groups preventing phosphorylation of the substrate.

40. (Previously presented) The composition of claim **36** or **306**, wherein one of the third label and the fourth label or the quencher is located at the C-terminus of the polypeptide and the other of the third label and the fourth label or the quencher is within the polypeptide.

41. (Previously presented) The composition of claim **36** or **306**, wherein the third and fourth labels are fluorophores capable of exhibiting FRET.

42. (Previously presented) The composition of claim **36** or **306**, wherein the phosphobinder is associated with one or more second caging groups, the presence of which prevents the phosphobinder from binding the phosphorylated second substrate.

43. (Original) The composition of claim **42**, wherein the second caging groups are removable under different conditions than the first caging groups preventing phosphorylation of the substrate and/or under different conditions than the third caging groups preventing phosphorylation of the second substrate.

44. (Previously presented) The composition of claim **36** or **306**, wherein the phosphobinder comprises an antibody, an SH-2 domain, a PTB domain, a 14-3-3 domain, an FHA domain, a WD40 domain and/or a WW domain.

45. (Previously presented) A composition, comprising:

a cell comprising an enzyme and a caged sensor for detecting an activity of the enzyme, which caged sensor comprises:

a) one or more molecules collectively comprising:

i) a substrate for the enzyme, wherein the substrate is in a first state on which the enzyme can act, thereby converting the substrate to a second state, and

ii) a first label, wherein a first signal exhibited by the first label when the substrate is in its first state is distinguishable from a second signal exhibited by the first label when the substrate is in its second state, and,

b) one or more first caging groups associated with the one or more molecules, the first caging groups inhibiting the enzyme from acting upon the substrate;

wherein the one or more molecules comprise a fifth label, the fifth label exhibiting a unique fifth signal, the fifth signal being independent of the state of the substrate.

46. (Previously presented) The composition of claim **45** or **307**, wherein the fifth label is a fluorophore or a quantum dot.

47-305. (Cancelled)

306. (Previously presented) A composition, comprising:

an enzyme and a caged sensor for detecting an activity of the enzyme, which caged sensor comprises:

a) one or more molecules collectively comprising:

i) a substrate for the enzyme, wherein the substrate is in a first state on which the enzyme can act, thereby converting the substrate to a second state, wherein the first state is not converted to the second state by cleavage by the enzyme, and

ii) a first label, wherein a first signal exhibited by the first label when the substrate is in its first state is distinguishable from a second signal exhibited by the first label when the substrate is in its second state; and,

b) one or more first caging groups associated with the one or more molecules, the first caging groups inhibiting the enzyme from acting upon the substrate;

wherein the enzyme is a protein kinase that phosphorylates tyrosine, serine and/or threonine;

wherein the one or more molecules comprise one polypeptide; wherein the one polypeptide comprises the substrate for the kinase, a second substrate, the first label, a third label, a fourth label or a quencher, and a phosphobinder; the substrate comprising a serine, threonine, or tyrosine residue capable of being phosphorylated by the kinase; the second substrate being associated with one or more third caging groups, the presence of which

prevents phosphorylation of the second substrate; wherein the first label is located at the serine, threonine, or tyrosine residue and exhibits the first signal when the residue is not phosphorylated and the second signal when the residue is phosphorylated; wherein the third label and the fourth label or the quencher do not interact when the second substrate is not phosphorylated, thereby producing a third signal; and wherein phosphorylation of the second substrate results in intramolecular binding of the phosphobinder to the phosphorylated second substrate, the intramolecular binding resulting in the interaction of the third label and the fourth label or the quencher, thereby producing a fourth signal, the fourth signal distinguishable from the first, second and third signals.

307. (Previously presented) A composition, comprising:

an enzyme and a caged sensor for detecting an activity of the enzyme, which caged sensor comprises:

a) one or more molecules collectively comprising:

i) a substrate for the enzyme, wherein the substrate is in a first state on which the enzyme can act, thereby converting the substrate to a second state, wherein the first state is not converted to the second state by cleavage by the enzyme, and

ii) a first label, wherein a first signal exhibited by the first label when the substrate is in its first state is distinguishable from a second signal exhibited by the first label when the substrate is in its second state, and,

b) one or more first caging groups associated with the one or more molecules, the first caging groups inhibiting the enzyme from acting upon the substrate;

wherein the one or more molecules comprise a fifth label, the fifth label exhibiting a unique fifth signal, the fifth signal being independent of the state of the substrate.

308. (Cancelled)

309. (Currently amended) A composition, comprising:

an enzyme and a caged sensor for detecting an activity of the enzyme, which caged sensor comprises:

a) one or more molecules collectively comprising:

i) a substrate for the enzyme, wherein the substrate is in a first state on which the enzyme can act, thereby converting the substrate to a second state, wherein the first state is not converted to the second state by cleavage by the enzyme, and

ii) a first label, wherein a first signal exhibited by the first label when the substrate is in its first state is distinguishable from a second signal exhibited by the first label when the substrate is in its second state; ~~and~~,

b) one or more first caging groups associated with the one or more molecules, the first caging groups inhibiting the enzyme from acting upon the substrate; and,

c) optionally, one or more second caging groups associated with a phosphobinder, wherein the presence of the ~~phosphobinder~~ second caging groups prevents the phosphobinder from binding the phosphorylated substrate;

wherein the enzyme is a protein kinase that phosphorylates tyrosine, serine and/or threonine;

wherein the one or more molecules comprise one polypeptide; wherein the one polypeptide comprises the substrate for the kinase and the first label and comprises a second label or a quencher; wherein the first label and the second label or the quencher interact to produce the first signal when the substrate is not phosphorylated; and wherein phosphorylation of the substrate prevents the interaction of the first label and the second label or the quencher, thereby resulting in production of the second signal;

wherein phosphorylation of the substrate triggers a conformational change in the polypeptide, the conformational change preventing the interaction of the first label and the second label or the quencher; or wherein phosphorylation of the substrate results in binding of a ~~the~~ phosphobinder to the phosphorylated substrate, the binding of the phosphobinder preventing the interaction of the first label and the second label or the quencher.

310. (Currently amended) A composition, comprising:

an enzyme and a caged sensor for detecting an activity of the enzyme, which caged sensor comprises:

a) one or more molecules collectively comprising:

i) a substrate for the enzyme, wherein the substrate is in a first state on which the enzyme can act, thereby converting the substrate to a second state, wherein the first state is not converted to the second state by cleavage by the enzyme, and

ii) a first label, wherein a first signal exhibited by the first label when the substrate is in its first state is distinguishable from a second signal exhibited by the first label when the substrate is in its second state; ~~and~~,

b) one or more first caging groups associated with the one or more molecules, the first caging groups inhibiting the enzyme from acting upon the substrate; and,

c) optionally, a phosphobinder comprising: an antibody, an SH-2 domain, a PTB domain, a 14-3-3 domain, an FHA domain, a WD40 domain and/or a WW domain;

wherein the enzyme is a protein kinase that phosphorylates tyrosine, serine and/or threonine;

wherein the one or more molecules comprise one polypeptide; wherein the one polypeptide comprises the substrate for the kinase and the first label and comprises a second label or a quencher; wherein the first label and the second label or the quencher interact to produce the first signal when the substrate is not phosphorylated; and wherein phosphorylation of the substrate prevents the interaction of the first label and the second label or the quencher, thereby resulting in production of the second signal;

wherein phosphorylation of the substrate triggers a conformational change in the polypeptide, the conformational change preventing the interaction of the first label and the second label or the quencher; or wherein phosphorylation of the substrate results in binding of the phosphobinder to the phosphorylated substrate, the binding of the phosphobinder preventing the interaction of the first label and the second label or the quencher.

311. (Currently amended) A composition, comprising:

an enzyme and a caged sensor for detecting an activity of the enzyme, which caged sensor comprises:

a) one or more molecules collectively comprising:

i) a substrate for the enzyme, wherein the substrate is in a first state on which the enzyme can act, thereby converting the substrate to a second state, wherein the first state is not converted to the second state by cleavage by the enzyme, and

ii) a first label, wherein a first signal exhibited by the first label when the substrate is in its first state is distinguishable from a second signal exhibited by the first label when the substrate is in its second state; ~~and~~,

b) one or more first caging groups associated with the one or more molecules, the first caging groups inhibiting the enzyme from acting upon the substrate; and,

c) one or more second caging groups associated with a phosphobinder, wherein the presence of the ~~phosphobinder~~ second caging groups prevents the phosphobinder from binding ~~on~~ the phosphorylated substrate;

wherein the enzyme is a protein kinase that phosphorylates tyrosine, serine and/or threonine;

wherein the one or more molecules comprise one polypeptide; wherein the one polypeptide comprises the substrate for the kinase and the first label; wherein the polypeptide comprises the phosphobinder and a second label or a quencher; wherein the first label and the second label or the quencher do not interact when the substrate is not phosphorylated, thereby producing the first signal; and wherein phosphorylation of the substrate results in intramolecular binding of the phosphobinder to the phosphorylated substrate, the intramolecular binding resulting in the interaction of the first label and the second label or the quencher, thereby producing the second signal; or, wherein the one or more molecules comprise a first polypeptide and a second polypeptide; wherein the first polypeptide comprises the substrate for the kinase and the first label; wherein the second polypeptide comprises the phosphobinder and a second label or a quencher; wherein the first label and the second label or the quencher do not interact when the substrate is not phosphorylated, thereby producing the first signal; and wherein phosphorylation of the substrate results in intermolecular binding of the phosphobinder to the phosphorylated substrate, the

intermolecular binding resulting in the interaction of the first label and the second label or the quencher, thereby producing the second signal.

312. (Previously presented) A composition, comprising:

an enzyme and a caged sensor for detecting an activity of the enzyme, which caged sensor comprises:

a) one or more molecules collectively comprising:

i) a substrate for the enzyme, wherein the substrate is in a first state on which the enzyme can act, thereby converting the substrate to a second state, wherein the first state is not converted to the second state by cleavage by the enzyme, and

ii) a first label, wherein a first signal exhibited by the first label when the substrate is in its first state is distinguishable from a second signal exhibited by the first label when the substrate is in its second state; and,

b) one or more first caging groups associated with the one or more molecules, the first caging groups inhibiting the enzyme from acting upon the substrate;

wherein the enzyme is a protein kinase that phosphorylates tyrosine, serine and/or threonine;

wherein the one or more molecules comprise one polypeptide; wherein the one polypeptide comprises the substrate for the kinase and the first label; wherein the polypeptide comprises a phosphobinder and a second label or a quencher; wherein the first label and the second label or the quencher do not interact when the substrate is not phosphorylated, thereby producing the first signal; and wherein phosphorylation of the substrate results in intramolecular binding of the phosphobinder to the phosphorylated substrate, the intramolecular binding resulting in the interaction of the first label and the second label or the quencher, thereby producing the second signal; or, wherein the one or more molecules comprise a first polypeptide and a second polypeptide; wherein the first polypeptide comprises the substrate for the kinase and the first label; wherein the second polypeptide comprises a phosphobinder and a second label or a quencher; wherein the first label and the second label or the quencher do not interact when the substrate is not phosphorylated, thereby producing the first signal; and wherein phosphorylation of the substrate results in

intermolecular binding of the phosphobinder to the phosphorylated substrate, the intermolecular binding resulting in the interaction of the first label and the second label or the quencher, thereby producing the second signal;

wherein the phosphobinder comprises an antibody, an SH-2 domain, a PTB domain, a 14-3-3 domain, an FHA domain, a WD40 domain and/or a WW domain.